Engineering the spatial organization of metabolic pathways

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Engineering the Spatial Organization of Metabolic Pathways

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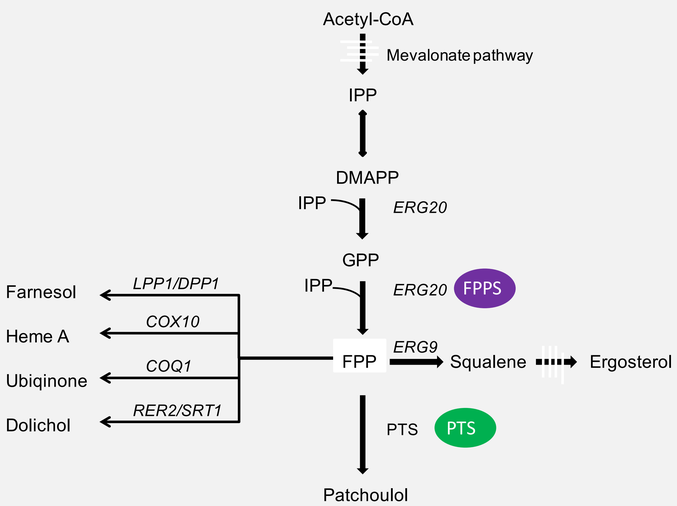
Abstract

Several natural systems for ensuring optimal spatial arrangement of biosynthetic enzymes exist. Sequentially acting enzymes can for example be positioned in close proximity by attachment to cellular structures, up-concentration in membrane enclosed organelles or assembly into large complexes. The vision is that by positioning sequentially acting enzymes in close proximity, the cell can accelerate reaction rates and thereby prevent loss of intermediates through diffusion, degradation or competing pathways. The production of valuable metabolites in cell factories often depends on both heterologous and host enzymes. In this case, no spatial coordination of the biosynthetic enzymes can be expected to be in place. Presumably this contributes to the low productivity regularly observed for heterologous pathways. In one test case, we investigated whether a heterologous pathway could be optimized by positioning two sequentially acting enzymes in close proximity. More specifically, we fused patchoulol synthase originating from Patchouli (*Pogostemon Cablin*) to the natural yeast enzyme, farnesyldiphosphate synthase (FPPS) and expressed it in the well-characterised cell factory *Saccharomyces cerevisiae*. Successfully, the sesquiterpene production was increased two-fold when the enzymes were fused compared to when they were expressed from the same promoters as free enzymes. Moreover, the strategy could be used in combination with other traditional metabolic engineering strategies to increase the production of a desired product, as enzyme fusion combined with down-regulation of a competing pathway and up-regulation of a selected pathway enzyme resulted in a five-fold higher sesquiterpene production. This simple test case demonstrates that engineering of the spatial organization of pathways has great potential for diverting flux towards a desired product.

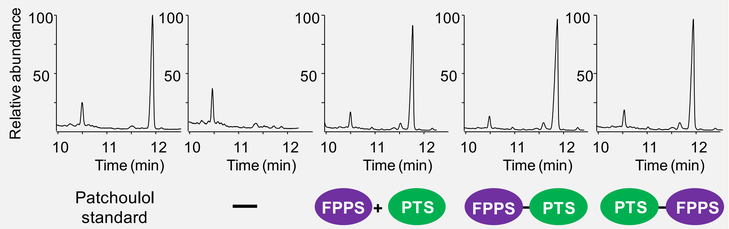
Pathway



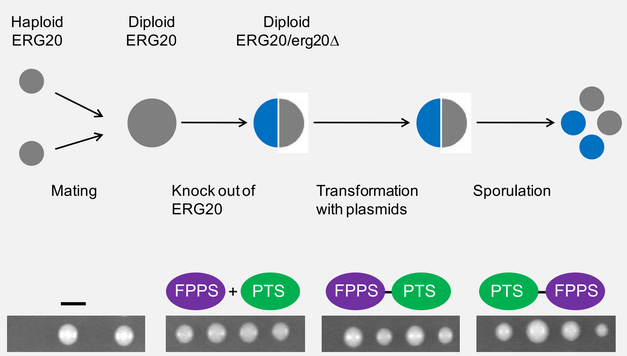
Functionality

Is PTS functional when fused to FPPS?

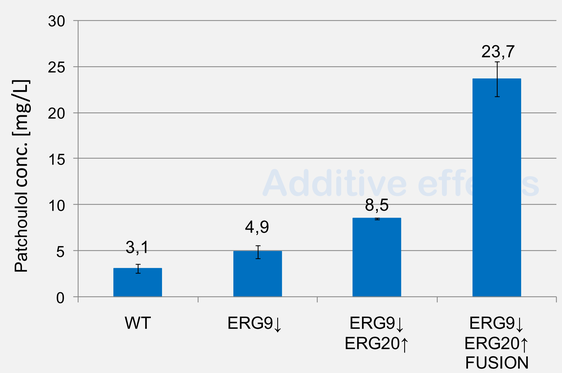
Results of GC-MS analysis:



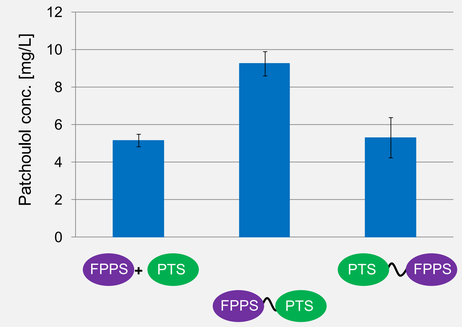
Is FPPS functional when fused to PTS?



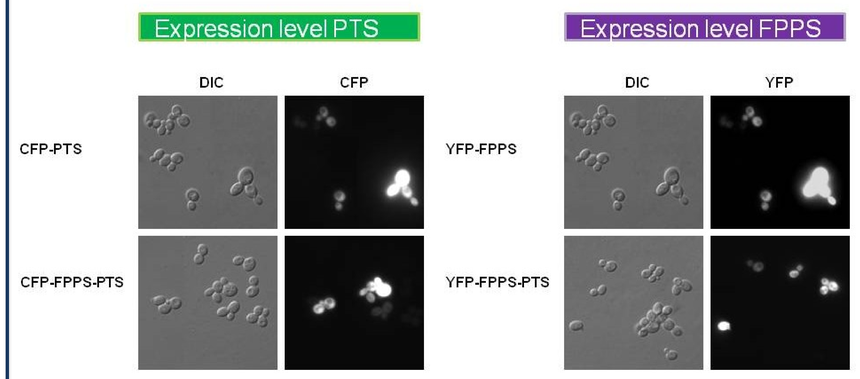
Additive effects



Effect of enzyme fusion



Expression levels



Effect of linker